Mass spectrometry: from imaging to metabolic networks

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Abstract

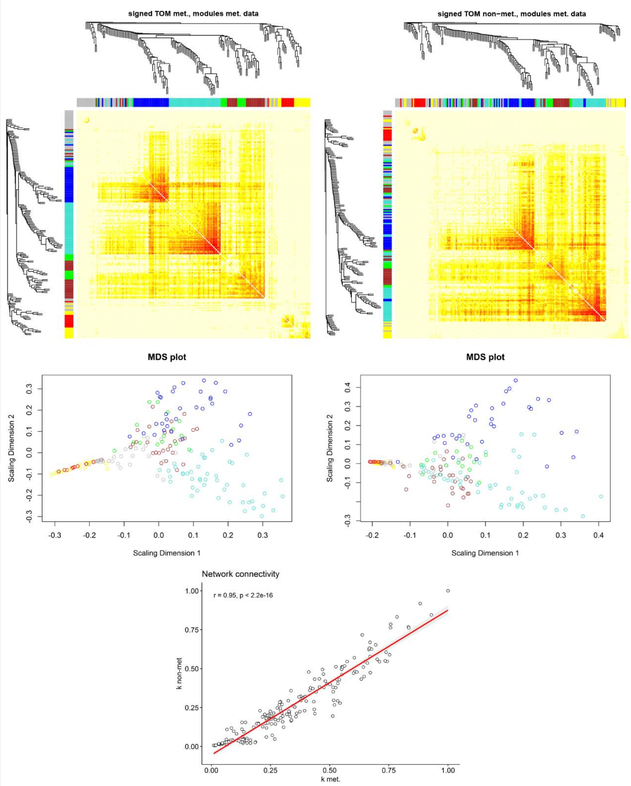
A deeper understanding of inter-tumor and intra-tumor heterogeneity is a critical factor for the advancement of next generation strategies against cancer. Under the hypothesis that heterogeneous progression of tumors is mirrored by their metabolic heterogeneity, detection of biochemical mechanisms responsible of the local metabolism becomes crucial.
We show that network analysis of co-localized ions from mass spectrometry imaging data provides a detailed chemo-spatial insight into the metabolic heterogeneity of tumor. Furthermore, module preservation analysis between colorectal cancer patients with and without metastatic recurrence suggests hypotheses on the nature of the different local metabolic pathways.

Introduction

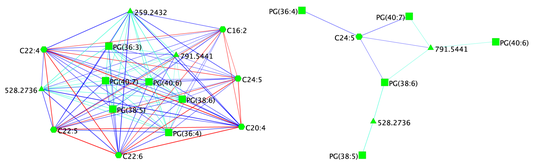
Mass spectrometry imaging (MSI) represents a powerful tool for the identification of molecular spatial distributions in biological specimens. Current standard techniques, such as MALDI, DESI and SIMS, have proved to be capable to detect mass spectral signatures associated with cancerous tissue. The large amount of extracted information, however, represents one of the most challenging aspects in the understanding of the underlying biochemical mechanisms associated with the observed different molecular distributions. For this scope, network analysis represents a natural choice, since it is capable to elegantly generate models of ion co-localization patterns that can be interpreted as possible representations of the local metabolic pathways.
Under such assumption, network analysis models suggest hypotheses on the biochemical interactions responsible of the observed co-localization patterns. The additional spatial dimension, characteristic of MSI, provide a further test for the plausibility of the detected patterns, when compared with the morphological properties of the tissue. Furthermore, differences between groups of patients in terms of these patterns can be used to generate hypotheses on the mechanisms responsible of the different observed clinical outcomes.

Materials & Methods

Weighted co-expression gene network analysis (WGNCA)[1] was applied to detect the metabolic differences between two groups of patients (e.g. patients with or without metastatic relapse). A consensus network is defined using the signed adjacencies from MSI data of each sub-cohort patients' MSI data. Afterwards, module detection is applied to the topological overlap matrix, in order to identify highly co-localized subsets of ions. Finally, modules with different network topologies between the two sub-cohort networks are detected using a module preservation analysis[2,3] (based on a permutation test). A null hypothesis of modules independence from patients' status is performed running N=100 times the entire analysis on 2 networks generated after shuffling the patients' sub-cohort membership labels. A cohort of 32 colorectal cancer patients was tested for determining metabolic differences between patients with and without metastatic relapse (in a follow-up period of up to 5 years after the surgical removal). The tested cohort consisted of 8 patients with metastatic relapse and 24 patients without a metastatic relapse.



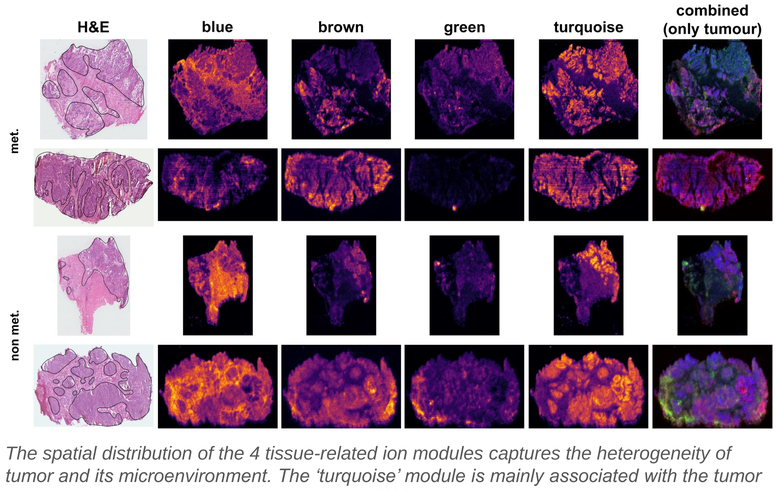
Network adjacency heatmaps for the metastatic (left) and non-metastatic (right) patients. A hierarchical clustering dynamic cut tree algorithm identified 6 modules of highly co-localized ions. 2-dimensional MDS scatter plot shows that the metastatic network module labels (represented by different colors) are consistent with the similarity between ion patterns. Furthermore, the high correlation between the connectivity of the two networks confirms their global similarity.



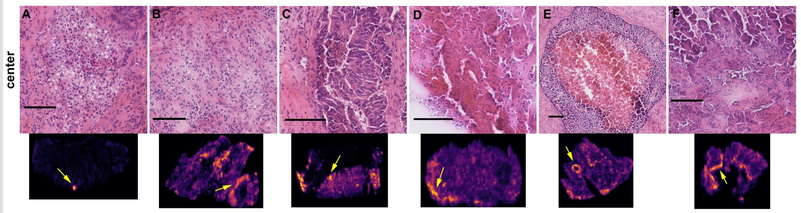
The unpreserved metastatic network module in the non-metastatic network consists of phospatidylglycerols (PG) and PUFAs. The presence of Arachidonic acid (C20:4) in the selected module suggests the hypothesis that a inflammatory related mechanism is responsible of the occurrence of a metastatic relapse in the analyzed patients.

Results

WGNCA applied to DESI-MSI[4] (negative ion mode) data from specimens collected at the center of the tumor revealed the presence of 6 ion modules in the metastatic-related network.
The module preservation analysis revealed that one of the 4 tissue-related metastatic network modules was not preserved in the non-metastatic network.
The presence of PGs and Arachidonic acid in the unpreserved module suggests that this module represents a local activity of phospholipase A2, related with an inflammatory response of the host. This is confirmed by the visual inspection of the associated spatial regions in the optical images of the H&E stained tissues. Free lipid droplets and macrophages were observed in those regions, suggesting a local inflammatory condition that has previously associated with an increased risk tumor infiltration[5].



The spatial distribution of the 4 tissue-related ion modules captures the heterogeneity of tumor and its microenvironment. The 'turquoise' module is mainly associated with the tumor tissue (delineated in the optical image of the H&E stained tissue).



The spatial distribution of the unpreserved module ions in 6 examples of metastatic (A-B-C) and non-metastatic (D-E-F) samples is compared with the H&E stained tissue. In the metastatic samples, the module corresponds to the presence of macrophages and lipids droplets, whereas in the non-metastatic corresponds to necrotic tissue.

Conclusion

For the first time, we show that network analysis, in particular WGCNA, can be employed for exploratory analysis in mass spectrometry imaging data.
The application of WGCNA efficiently reveals patterns of co-localized ions and their spatial distributions through their eigen-metabolite (analogous to the standard eigen-gene vectors). The assumption that co-localized ions represent the different local metabolic pathways is exploited here to make hypothesis on the biochemical mechanisms associated with tumor heterogeneity. Furthermore, the module preservation analysis allows the identification of groups of co-localized ions in patients with metastatic recurrence that do not occur in the non-metastatic patients. This technique is used to identify hypotheses on the different local metabolism that can be associated with the different clinical outcome.
The presented method can be easily extended to datasets from other tissue types.

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Datasets and scripts are publicly available at https://doi.org/10.4121/uuid:f06dee0d-1d2e-4d67-b978-5bac4087d346.

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